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Clinical variability in late-AO cases of transthyretin related Familial Amyloid Polyneuropathy diagnosed from 2007 to 2016

Ana do Carmo Ramalho Moreira Azevedo

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Autora: Ana do Carmo Ramalho Moreira Azevedo

Afiliação: Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto

Endereço: Rua de Jorge Viterbo Ferreira nº 228, 4050-313 Porto

Correio eletrónico: anaramalhoazevedo@gmail.com

Orientadora: Professora Doutora Ana Martins da Silva

Assistente Hospitalar Graduada de Neurologia do Centro Hospitalar do Porto

Professora auxiliar convidada do MIM do ICBAS/CHP

Coorientadora: Professora Doutora Carolina Lemos

Professora auxiliar convidada do MIM do ICBAS/CHP

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Ana Azevedo

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LIST OF ABBREVIATIONS

AO	Age at onset
FAP	Familial Amyloid Polyneuropathy
IVS	Interventricular Septum
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
mPND	Modified polyneuropathy disability
TTR	Transthyretin
TTR-FAP	Transthyretin familial amyloid polyneuropathy

ABSTRACT

Introduction: Patients with transthyretin familial amyloid polyneuropathy were first described in geographically restricted areas, but progressively more and more patients were identified outside these disease foci, with genetic and clinical heterogeneity and variable age at onset.

Aims: The main goal of this project is to characterize late-onset transthyretin familial amyloid polyneuropathy patients from the North and Center of Portugal, about clinical manifestations at the diagnosis, demographics and genetic data.

Methods: A retrospective study was performed involving 111 patients with late-onset familial amyloid polyneuropathy, diagnosed from 2007 to 2016, at Unidade Corino de Andrade - Centro Hospitalar do Porto. Patients' files were accessed and data concerning demographics, family history, diagnosis delay, clinical manifestations, neurological evaluation, cardiac examination indices and laboratory findings at diagnosis were collected.

Results: Patients belong to 93 families (51 from the Center of Portugal, 41 from the North and 1 from Lisboa and Vale do Tejo), fifty-one (54.8%) of them with family history. The disease in these patients showed: a male preponderance (1.5:1, more evident in the group without family history – 3.7:1); a mean age at onset of 61.7 ± 8.4 years (higher in male patients - 63.1±7.9 vs 59.6±8.7 years, p=0.0034); sensory neuropathy in 95.5% and motor neuropathy in 45.9%; on neurological examination, 40.3% had sensory dissociation and 24.3% a modified Polyneuropathy Disability score≥3; dysautonomia was present in 90.1% of patients; myocardiopathy was more evident in male patients and heart failure was present in 15 patients; only female patients had signs of severe renal dysfunction.

Discussion: This study confirmed some of the conclusions of Japanese authors, including the dispersed distribution of these late-onset cases, the male predominance, the description of previous cases in families and the severe myocardiopathy. However, unlike Japanese studies, these patients had evident dysautonomia and sensory dissociation. We also confirmed the severe renal dysfunction in female patients.

Keywords: familial amyloid polyneuropathy, transthyretin, late-onset Portuguese patients, clinical variability

RESUMO

Introdução: A polineuropatia amiloidótica familiar foi inicialmente descrita em áreas geográficas restritas. Nos últimos anos, cada vez mais doentes, com maior heterogeneidade clínica, genética e variabilidade na idade de início, foram sendo identificados fora destes focos de doença.

Objetivo: O principal objetivo deste projeto foi caracterizar os doentes com polineuropatia amiloidótica familiar de início tardio, do Norte e Centro de Portugal, quanto às manifestações clínicas ao diagnóstico e aos dados demográficos e genéticos.

Doentes e métodos: Foi realizado um estudo retrospetivo com 111 doentes com polineuropatia amiloidótica familiar de início tardio, diagnosticados entre 2007 e 2016, na Unidade Corino de Andrade do Centro Hospitalar do Porto. Foram recolhidos dos registos clinicos os seguintes dados: idade, sexo, idade do diagnóstico, história familiar, dados registados em consultas médicas, exame neurológico, avaliação cardiovascular e resultados analíticos.

Resultados: Os doentes pertenciam a 93 famílias (51 do Centro de Portugal, 41 do Norte e uma de Lisboa e Vale do Tejo), das quais 51 doentes (54.8%) tinham história familiar. Neste grupo de doentes de início tardio encontramos predomínio de homens (1.5:1, sendo maior no grupo sem história familiar 3.7:1); a média de idade de início de doença foi 61.7 ± 8.4 anos (sendo maior nos homens 63.1±7.9 vs 59.6±8.7 anos, p=0.034); neuropatia sensitiva em 95.5% e neuropatia motora em 45.9%; ao exame neurológico, 40.3% apresentaram dissociação sensitiva e 24.3% com *modified Polyneuropathy Disability score*≥3; disautonomia em 90.1%; miocardiopatia mais evidente nos homens; insuficiência cardíaca em 15 doentes; e sinais de disfunção renal grave apenas em mulheres.

Discussão: Este estudo confirmou algumas das conclusões dos autores Japoneses relativas à doença de início tardio, incluindo a distribuição mais dispersa destes doentes, o predomínio de casos em homens, a presença de história familiar e de cardiomiopatia. No entanto, ao contrário dos estudos Japoneses, os doentes apresentam disautonomia e dissociação sensitiva. Confirmámos também a presença de disfunção renal grave apenas em mulheres.

Palavras-chave: polineuropatia amiloidótica familiar, transtirretina, doentes Portugueses de início tardio, variabilidade clínica

INTRODUCTION

Familial amyloid polyneuropathy (FAP) is an autosomal-dominant, adult-onset disorder, with phenotypic and genotypic heterogeneity. Transthyretin familial amyloid polyneuropathy (TTR-FAP) is associated with over 100 different mutations in the transthyretin (*TTR*) gene, including the most common mutation methionine-for-valine substitution at position 30 (Val30Met) (Andrade, 1952, Saraiva et al., 1984, Araki and Ando, 2010). These pathogenic mutations lead to protein misfolding with formation of amyloid fibrils and, ultimately, amyloid deposition in tissues (predominantly in the peripheral nervous system, somatic and autonomic, and the heart) (Figure 1) (Merlini and Bellotti, 2003, Hou et al., 2007).

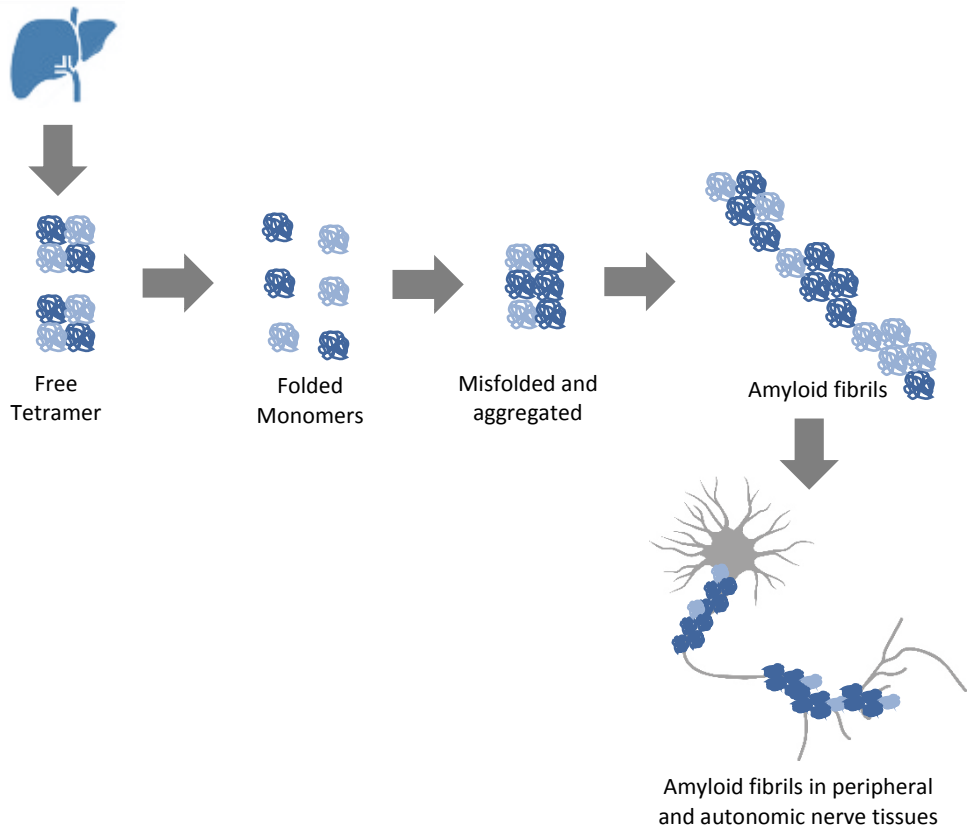

Figure 1: Amyloid fibril formation in transthyretin familial amyloid polyneuropathy. The pathogenic mutations decrease the stability of transthyretin tetramers and increase their dissociation into monomers, which self-assemble in the extracellular space, leading to the formation of non-fibrillar soluble oligomers and protofibrils that assemble to create insoluble amyloid fibrils deposits in peripheral and autonomic nerve tissues in later stages of disease (Merlini and Bellotti, 2003, Hou et al., 2007).

Patients with TTR-FAP were first described in geographically restricted areas of Sweden, Japan and Portugal (Andrade, 1952, Andersson, 1976, Ikeda et al., 1987), but progressively more and more patients were identified outside these disease foci, with genetic heterogeneity and variable age at onset (AO) (Parman et al., 2016).

In Portugal, Andrade's first description presented a disease of young adults (Andrade, 1952) but since then more and more late-onset cases were diagnosed, disclosing a wide variation in AO from 19 to 82 years of age (Sousa et al., 1995). Besides, several aged-asymptomatic gene carriers, up to 95 years, were identified, suggesting a variable penetrance of the gene (Sousa et al., 1995).

Classical TTR-FAP was characterized as a disease of young adults with a positive family history, originated from localized regions, the disease foci, presenting a severe progressing mixed neuropathy that shows early involvement of small nerve fibers (Coutinho, 1980). The presenting symptoms are commonly neuralgic pain, loss of temperature and pain sensation and autonomic manifestations, including erectile dysfunction, bladder paresis, gastroparesis, constipation, diarrhea, sweating abnormalities and postural hypotension (Figure 2) (Coutinho, 1980, Conceicao et al., 2016). Untreated neuropathy progresses rapidly with motor problems and loss of postural sensation. Other manifestations are weight loss, cardiac arrhythmia, renal failure, proteinuria and vitreous opacities (Coutinho, 1980, Conceicao et al., 2016). The terminal manifestations of the disease are severe weakness, marked trophic changes, dysphagia, and urinary and fecal incontinence (Coutinho, 1980, Conceicao et al., 2016).

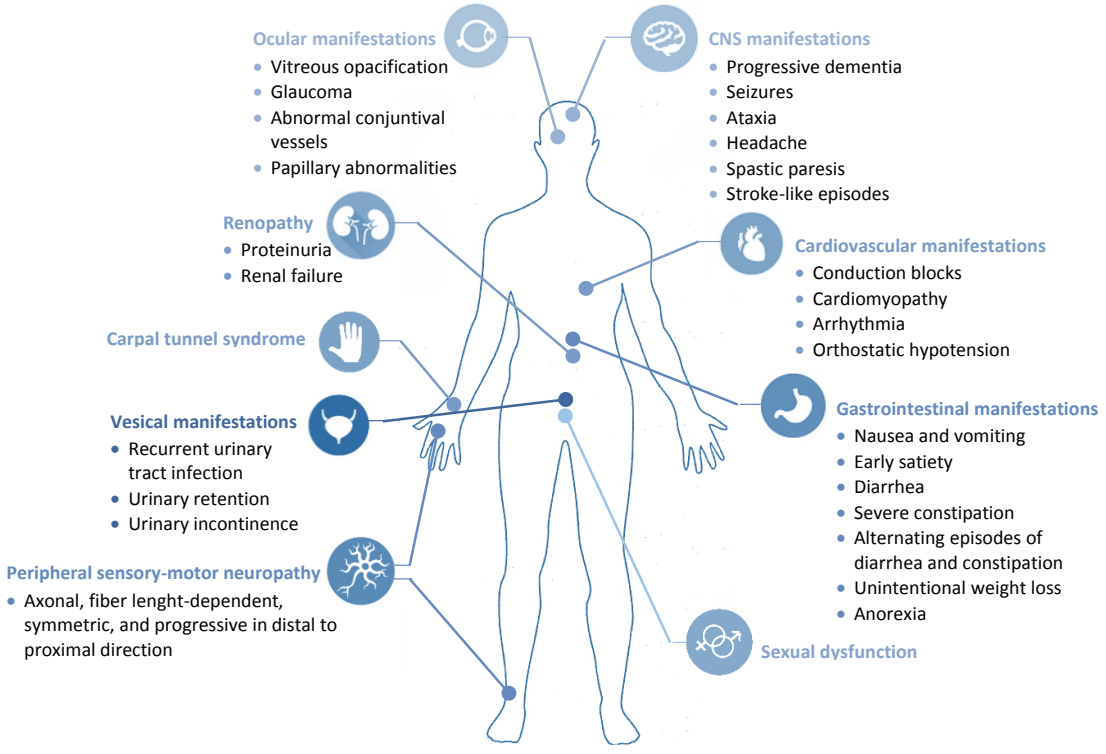

Figure 2: Clinical features associated with transthyretin familial amyloid polyneuropathy (CNS: Central Nervous System) (Adapted from Conceicao et al., 2016).

In the last two decades Japanese authors called the attention to the differences between patients with early-AO (< 50 years of age) and late-AO (≥ 50 years of age). They stated that early and late-onset patients are two distinct groups. Early-onset patients are related to endemic foci where patients follow the typical description of the disease. In contrast, late-onset patients have a sporadic presentation due to low penetrance of the gene, come from non-endemic regions with no relation to disease foci and are characterized by relative preservation of unmyelinated nerve fibers and axonal sprouting. Consequently, these patients have impaired superficial and deep sensation, early distal motor loss and relatively mild autonomic symptoms associated with a severe cardiomyopathy (Sobue et al., 2003, Koike et al., 2004, Koike et al., 2011, Koike et al., 2012).

Genotype heterogeneity, geographical origin of patient, penetrance of the gene mutation and AO, contribute to the wide variation in clinical presentation of TTR-FAP (Ando et al., 1993, Adams et al., 2012a, Koike et al., 2012, Adams et al., 2014). This wide spectrum of phenotypes makes difficult to recognize an index case of TTR-FAP and accurate diagnosis is often delayed for years. Delay in diagnosis is most pronounced in regions where TTR-FAP is not common and when there is no positive family history because there is a lack of awareness among physicians of that region, leading to higher rates of misdiagnosis and poorer patient outcomes (Plante-Bordeneuve et al., 2007, Koike et al., 2011, Adams et al., 2012a, Dohrn et al., 2013). Given the limited window for treatment effectiveness and the fast and irreversible tissue damage, an early and accurate diagnosis is essential to enable effective therapeutic intervention in early disease stages and better prognosis (Coelho et al., 2013, Plante-Bordeneuve, 2014, Ericzon et al., 2015).

The main goal of this project is to assess clinical and genetic features of late-AO TTR-FAP patients from the North and Center of Portugal. For this purpose, all late-onset patients diagnosed from 2007 to 2016 at Unidade Corino de Andrade - Centro Hospitalar do Porto, were characterized about the geographic origin, family history and the presenting symptoms at the diagnosis (length-dependent sensory-motor polyneuropathy, autonomic dysfunction, cardiac manifestations, ocular symptoms and renal dysfunction).

PATIENTS AND METHODS

Patients and Methods

A retrospective study was performed involving 111 patients with late-AO FAP (108 Val30Met and 3 Val28Met), diagnosed from 2007 to 2016, at Unidade Corino de Andrade - Centro Hospitalar do Porto. The inclusion criteria were 1) Patients with TTR-FAP mutations identified by deoxyribonucleic acid analysis of blood leukocytes; 2) symptom or sign unequivocally associated with TTR-FAP; 3) late-onset TTR-FAP (defined as clinical manifestations of TTR-FAP at ages over 50 years old) (Sequeiros and Saraiva, 1987, Misu et al., 1999, Koike et al., 2002).

Patients' files were accessed and data concerning demographics, clinical manifestations and results of complementary exams at the moment of diagnosis were collected. Diagnosis delay, defined as the interval from the initial manifestations related to FAP to the diagnosis of the disease, was also evaluated. In addition, family history and geographical distribution were assessed.

Patients were classified as symptomatic after a complete routine evaluation that included neurological clinical assessment by an experienced neurologist, electrocardiogram, echocardiogram, and blood tests (peripheral blood cell count, renal function tests, including urinary protein content, creatinine clearance and NT-proBNP). When visual symptoms were present, ophthalmologic examination was performed.

The symptoms and signs at presentation were categorized as elucidated in Table I: neuropathic symptoms, including sensory (paresthesia, numbness or pain) and motor symptoms (distal weakness); autonomic dysfunction, which consists of digestive symptoms (diarrhea, constipation, gastroparesis, nausea and vomiting), genito-urinary symptoms (sexual dysfunction, urinary incontinence, urinary tract infections and voiding difficulty or retention), cardiac symptoms or signs (syncope, palpitations, dizziness and orthostatic hypotension, defined as a decrease in systolic blood pressure of 20 mmHg or a decrease in diastolic blood pressure of 10 mmHg within three minutes of standing compared with blood pressure from the sitting or supine position (Freeman, 2016)) and xerophthalmia; ocular abnormalities, including vitreous opacities and glaucoma; and important weight loss, defined as loss of more than 10 percent of usual body weight over 6 to 12 months (Collins, 2003).

Table I: Signs and symptoms categories.

Signs and symptoms		
Neuropathic symptoms	Sensory symptoms	Paresthesia
		Numbness
		Pain
	Motor symptoms	Distal weakness
Autonomic dysfunction	Digestive symptoms	Diarrhea
		Constipation
		Gastroparesis
		Nausea
		Vomiting
	Genito-urinary manifestations	Sexual dysfunction
		Urinary incontinence
		Urinary tract infection
		Voiding difficulty and retention
	Cardiac symptoms or signs	Syncope
		Palpitations
		Dizziness
		Orthostatic hypotension
	Xerophthalmia	
Ocular abnormalities	Vitreous opacities	
	Glaucoma	
Important weight loss		

Neurological examination

Neurological examination was performed. Sensory dissociation was defined by reduced superficial sensation (nociception) along with preserved vibratory sensation.

The modified polyneuropathy disability score (mPND score) was calculated (Table II). Onset of the disease was defined by a neurologist specialized in the disease as the occurrence of the first progressive characteristic symptoms, coincident with an abnormal neurological observation or signs of other organ involvement.

Table II: Modified polyneuropathy disability score (mPND score) (Adams et al., 2015).

mPND Score	Definition
I	Sensory disturbance but preserved walking capacity.
II	Impaired walking capacity but ability to walk without stick or crutches.
IIIa	Walking with help of one stick or crutch.
IIIb	Walking with help of 2 sticks or crutches.
IV	Confined to wheelchair or bedridden.

Cardiac assessment

Increased interventricular septum (IVS) thickness (>11 mm) was used as a marker suggestive of cardiac amyloid deposition (Olofsson et al., 2002, Koike et al., 2011). The IVS thickness was measured by two-dimensional and M-mode echocardiography.

The presence of a decreased ejection fraction (≤40%) on echocardiography and an increased level of N-terminal prohormone of brain natriuretic peptide (NT-proBNP) were considered signs of cardiac failure (Koike et al., 2012, Ponikowski et al., 2016). NT-proBNP levels was categorized as above the upper limit of individual normal level and ≥900 pg/ml, a range with high positive predictive value for chronic heart failure in patients with ≥ 50 years (Januzzi et al., 2005).

Finally, electrocardiographic studies were analyzed for rhythm and conduction disturbances, including atrial fibrillation, bradycardia, atrial-ventricular and bundle branch blocks. Patients with implanted pacemaker had no electrocardiographic study.

Laboratory findings

Results from proteinuria were divided in four groups: 0.030-0.300 g of proteinuria on a spot urine protein-to-creatinine ratio, considered as microalbuminuria; >3.500 g of proteinuria on a spot urine protein–to-creatinine ratio, defined as nephrotic-range; and 2 intermediate groups (0.300-1.000 g and 1.000-3.500 g) (Lin and Denker, 2016).

The creatinine clearance was divided in 5 groups according to Kidney Disease Outcomes Quality Initiative classification of chronic kidney disease (Table III).

Levels of hemoglobin equal or lower than 12.0 g/dL were considered as anemia.

Table III: Classification of chronic kidney disease according to Kidney Disease Outcomes Quality Initiative (National Kidney, 2002).

Stage	Glomerular Filtration Rate (ml/min/1.73m^2)	Description
1	≥ 90	Normal or high kidney function.
2	60-89	Mildly reduced kidney function.
3	30-59	Moderately reduced kidney function.
4	15-29	Severely reduced kidney function.
5	< 15 or on dialysis	Very severe decreased kidney function or end-stage kidney failure.

Statistical analysis

Patient characteristics are reported as numbers and percentages for categorical variables and medians, with interquartile range, or means ± standard deviation for continuous variables. The t-Student test for independent samples and Mann-Whitney test were conducted as appropriate. A p-value of <0.05 was considered statistically significant. Statistical software used was IBM SPSS Statistics24.

This protocol was approved by the Comissão de Ética para a Saúde e Gabinete Coordenador de Investigação do Departamento de Ensino, Formação e Investigação do Centro Hospitalar do Porto.

RESULTS

Demographic and genetic features

Between 2007 and 2016, 111 cases of FAP with late-onset (67 men and 44 women; male:female ratio of 1.5:1), belonging to 93 unrelated families, were referred to Unidade Corino de Andrade. From these 93 families, 51 (54.8%) had been ascertained previously and registered at the center or diagnosed elsewhere and the remaining 42 families had no description of a similar disease in previous generations.

One hundred and eight patients (97.3%) had Val30Met variant of TTR and the remaining 3 patients had Val28Met variant of TTR. The mean AO was 61.7 ± 8.4 years, ranging from 50 to 80 years. Male patients had a mean AO significantly higher than female patients (63.1±7.9 vs 59.6±8.7 years, with p=0.034). For all patients mean diagnostic delay was 2.7±2.1 years, ranging from 0 to 10 years. The mean diagnostic delay was significantly higher in patients without family history as compared with patients with previous description of disease in their families (3.5±1.9 vs 2.3±2.1 years, p=0.003).

We also analyzed the AO variability and gender ratio considering only one patient from each family. Patients with positive family history had statistically significant lower AO when compared with patients without family history (58.7 ± 7.9 vs 65.4± 8.1 years, p=0.001). In the group with family history, there were 27 male patients and 24 female patients (ratio 1.1:1). However, in the group with negative family history, there were 33 male patients and only 9 female patients (ratio 3.7:1).

Geographic origin

The families involved in this study were from different origins: 51 families from central areas, 41 from the North of Portugal and 1 from Lisboa and Vale do Tejo (Figure 3).

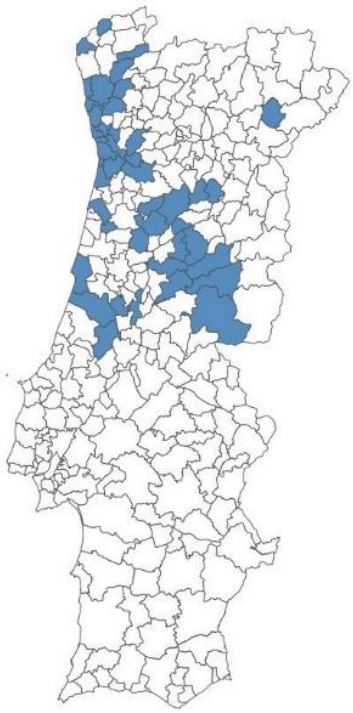

Figure 3: Distribution of families with late-onset cases of familial amyloid polyneuropathy in Portugal.

Clinical characteristics at diagnosis

Patients' clinical characteristics at the diagnosis are elucidated in Table IV. The most frequent symptoms were paraesthesia and/or neuralgic pain in the distal portion of lower limbs in 106 patients (95.5%), followed by distal weakness which was present in 51 patients (45.9%). Dysautonomia was detected in 100 patients (90.1%), with the majority of patients having digestive (70.3%) and cardiovascular (61.3%) symptoms. Glaucoma and/or vitreous opacities were seen in 6 patients (5.4%), and three of them had no neuropathic symptoms. Important weight loss was reported in 24 patients (21.6%).

Table IV: Initial clinical characteristics late-onset cases of transthyretin familial amyloid polyneuropathy.

Initial manifestations of TTR-FAP	Late-onset FAP ATTR (n=111) (%)
Neuropathic symptoms	107 (96.4)
Sensory symptoms	106 (95.5)
Motor symptoms	51 (45.9)
Autonomic symptoms	100 (90.1)
Gastro-intestinal symptoms	78 (70.3)
Genito-urinary symptoms	54 (48.6)
Cardiovascular abnormalities	68 (61.3)
Xerophtalmia	15 (13.5)
Ocular pathology (glaucoma or vitreous opacities)	6 (5.4)
Important weight Loss	24 (21.6)

Neurological examination at diagnosis

Complete and detailed sensory examination was available only for 72 patients. From these, sensory dissociation was present in 29 (40.3%) and severe loss of nociception and vibratory sensation was present in 36 (50.0%). Seven (9.7%) patients had no sensory neuropathy.

Polyneuropathy disability score (mPND score) was calculated: 6.3% of patients had no evidence of sensory or motor disturbances; 43.2% had only sensory disturbances; and, finally, for patients who had sensory and motor disturbances (50.5%), only 24.3% of the total required support for walking (Figure 4).

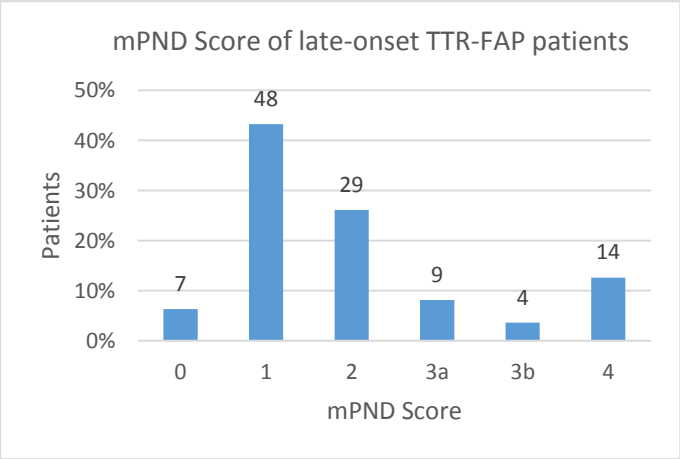

Figure 4: Modified Polyneuropathy Disability Score of 111 late-onset transthyretin familial amyloid polyneuropathy patients: mPND 0 – 6.3%; mPND 1 – 43.2%; mPND 2 – 26.1%; mPND 3a – 8.1%; mPND 3b – 3.6%; and mPND 4 – 12.6%.

Cardiac assessment

Patients' cardiac assessment is elucidated in Table V.

Levels of NT-proBNP were elevated in 49 of 107 (45.8%) patients; of those, 29 (27.1% of all patients) had levels above 900 pg/ml, a range with high positive predictive value for chronic heart failure. A decreased ejection fraction, a sign of heart failure, was observed in 3 of 82 (3.7%) patients. However, at the time of diagnosis, symptoms and signs of heart failure were reported in only fifteen of these cases.

On echocardiography, a thickened IVS was seen in 47 of 82 (57.3%) examined patients (Figure 5). The median of IVS thickness was 15.0 mm for male subjects and 13.0 mm for female patients, a significant statistically difference (p=0.005).

A pacemaker was implanted prior to the diagnosis of TTR-FAP in 9 patients (8.1%). Among the other 102 patients, the electrocardiographic study was performed in 98. Fourteen of the examined patients (14.3%) had sinus node dysfunction, 20 (20.4%) had atrioventricular conduction block and 39 (39.8%) had bundle branch block. In total 73 patients out of 98 (74.5%) had conduction disturbances.

Table V: Features on cardiac assessment of late-onset transthyretin familial amyloid polyneuropathy patients (IVS: interventricular septum).

Cardiac parameter assessed	Number of patients (%)
NT-proBNP (n=107) (relative and absolute frequencies)	
> upper limit of normal	49 (45.8)
>900 pg/ml	29 (27.1)
Echocardiogram (n=82) (relative and absolute frequencies)	
Reduced Ejection Fraction	3 (3.7)
Increased IVS thickness	47 (57.3)
Male (n=30)(median [interquartile range])(mm)	15.0 [11.7,18.3]
Female (n=12)(median [interquartile range])(mm)	13.0 [11.9,14.1]
Electrocardiographic study (n=98) (relative and absolute frequencies)	
Abnormal	73 (74.5)
Bradicardia/AF	14 (14.3)
Atrioventricular block	20 (20.4)
Bundle branch block	39 (39.8)
Pacemaker at presentation	9 (8.1)

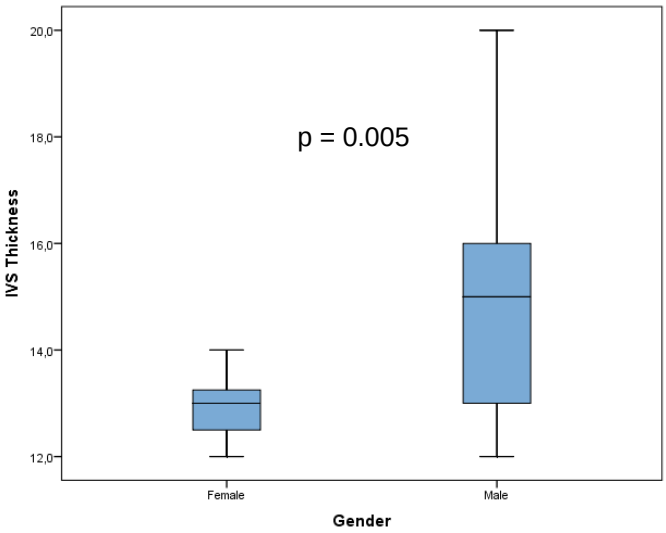

Figure 5: Interventricular septum thickness in late-onset male and female TTR-FAP patients (p=0.005).

Laboratory findings

Patient laboratory findings at the time of diagnosis are summarized in Table VI.

Normocytic and normochromic anemia was present in ten of 110 (9.0%) examined patients. The hemoglobin levels in these ten patients ranged from 9.0 g/dl to 11.7 g/dl.

Urine protein content was evaluated in 103 patients. Sixty patients had normal results. Twenty one patients (20.4%) had only microalbuminuria with normal renal function. Twenty patients presented non nephrotic degrees of proteinuria. The two patients with a nephrotic-range proteinuria had also severely decreased renal function. These and three patients that were on dialysis at the moment of the diagnosis were female.

Table VI: Laboratory findings at diagnosis of late-onset transthyretin familial amyloid polyneuropathy patients.

Laboratory parameter	Number of patients (%)
Hemoglobin (n=110)	
Mean ± Standard Deviation (g/dl)	13.8±1.4
< 12 g/dl (relative and absolute frequencies)	10 (9.0)
Creatinine Clearance (n=105) (relative and absolute frequencies)	
≥90 ml/min/1.73m^2	28 (26.7)
60-89 ml/min/1.73m^2	60 (57.1)
30-59 ml/min/1.73m^2	15 (14.3)
15-29 ml/min/1.73m^2	1 (1.0)
<15 ml/min/1.73m^2	1 (1.0)
Dialysis at presentation (n=111)	3 (2.7)
Urine Protein Content (n=103) (relative and absolute frequencies)	
0.030 – 0.300 g	21 (20.4)
0.300 – 1.000 g	12 (11.7)
1.000 – 3.500 g	8 (7.8)
>3.500 g	2 (1.9)

DISCUSSION

We assessed the clinical features, and demographic and genetic data of all Portuguese patients with late-onset TTR-FAP, diagnosed in the last decade in the reference center of Porto.

We aimed to compare late-onset Portuguese patients with previous descriptions of similar patients, namely the Japanese patients. In Japan, an important focus of early-onset disease with the Val30Met mutation was described in 1968 with clinical characteristics very similar to those described by Andrade in Portuguese patients (Araki et al., 1968). Molecular diagnosis available since 1984 made possible the identification of an increasing genetic heterogeneity and phenotypic variability (Saraiva et al., 1984). In the last two decades Japanese authors highlight the differences between early and late-onset patients considering that these patients can be divided in two clearly distinct groups (Sobue et al., 2003, Koike et al., 2012). According to their observations, late-onset patients originate from regions without any relation with the two well known endemic foci, in Nagano and Kumamoto districts, present very often as sporadic cases without previous family history, have a marked male predominance, show a neuropathy with early involvement of large nerve fibers, translated into the clinical presentation as a sensory and motor neuropathy without sensory dissociation and with minimal autonomic dysfunction and have also severe heart disease with infiltrative cardiomyopathy more than conduction disturbances (Araki et al., 1968, Sobue et al., 2003, Koike et al., 2012).

As previously described (Coelho et al., 1994), this study confirms that many but not all late-onset patients originate from regions where the disease is not frequent, showing a more dispersed distribution with only few cases coming from the districts with higher concentration of cases, including Póvoa de Varzim, Esposende, Vila do Conde, Barcelos and Figueira da Foz (Inês, 2015).

Family history is present in 54.8% of patients. This result is similar to that reported in Italian, Japanese and Swedish late-onset TTR-FAP population, with 33%, 48% and 65% of cases having family history, respectively (Suhr et al., 2009, Koike et al., 2012, Luigetti et al., 2013).

Male predominance is present, with a ratio male:female of 1.52:1 in the general sample. However if we only consider the group of sporadic probands the ratio is much higher (3.7:1). These results are similar to those described for late-onset patients from non-endemic regions, with this ratio varying from 4.36:1 to 6.14:1 (Sobue et al., 2003, Koike et al., 2012).

The analysis of the distribution of the AO showed a significant difference between patients with and without family history. The AO of male and female patients was also significantly different, with women presenting an earlier AO. This result is somehow

surprising because in the general population of Portuguese patients the AO has always been described as significantly lower in male patients but may be due to the relative concentration of female patients in the group with a positive family history that also shows an early AO (Lemos et al., 2014).

The majority of patients presented neuropathic symptoms, predominantly sensory disturbances (95.5%) while motor symptoms were less frequent, affecting 45.9% of examined patients. These results are similar to the experience in other countries with late-onset TTR-FAP, including Japan (Koike et al., 2012), Italy (Mariani et al., 2015), Sweden (Dohrn et al., 2013), France (Cappellari et al., 2011) and South of Portugal (Conceicao and De Carvalho, 2007). We identified sensory dissociation in 40.3% of the patients. However, many patients (50.0%) had a severe sensory loss that made impossible this differentiation and 9.7% had no sensory neuropathy. In general we may say that late-onset Portuguese patients do present sensory dissociation, a result strikingly different from French and Japanese series (Hornsten et al., 2010, Mariani et al., 2015).

Similarly to results reported in France, Portuguese late-onset TTR-FAP cases had a median mPND score at diagnosis of 2, however only 24.3% of patients required aid for walking at the time of diagnosis (mPND score 3 or 4), a different result from that of French late-onset patients, who need walking aid in 39% of the cases (Adams et al., 2012b). The higher number of patients with previous family history and a shorter diagnostic delay may contribute to this difference.

Autonomic dysfunction was present in the majority of patients (90.1%), a result that also distinguish these late-onset TTR-FAP patients from previous studies performed in Japan, Sweden, France, Germany and Italy, where the dysautonomia is less frequent, ranging from 10% in Japanese to 71% in Italian population (Cappellari et al., 2011, Koike et al., 2012, Dohrn et al., 2013, Mariani et al., 2015).

Ocular amyloidosis was seen in 5.4% of these patients, similarly to other late-onset patients from Japan, where this manifestation is present in 2-10% of late-onset patients (Koike et al., 2002, Koike et al., 2012). Curiously 3 of these patients had no signs of other organ involvement, as previously reported (Ciulla et al., 1995, Seca et al., 2014).

Amyloid deposition in the subendocardial layer of the myocardium, where the conduction system is located, explains the occurrence of cardiac conduction abnormalities and the frequent need for pacemaker implantation, frequently observed in early-onset patients. On the other hand, diffuse amyloid myocardial infiltration with ventricular wall thickening agrees with cardiac hypertrophy and subsequent heart failure in late-onset cases.

Electrocardiographic abnormalities were seen in 74.5% of the patients without pacemaker. The most common abnormality was bundle branch block, followed by atrioventricular block and sinus node dysfunction. A small group of patients (8.1%) needed pacemaker before diagnosis. A similar prevalence of conduction abnormalities was also verified in France and Sweden (Hornsten et al., 2010, Mariani et al., 2015).

In more than a half of patients, the IVS thickness was increased. In this analysis, male gender was clearly associated with more pronounced septal hypertrophy, similarly to findings reported for senile systemic amyloidosis and for late-onset TTR-FAP Swedish patients (Ng et al., 2005, Hornsten et al., 2010). This finding is in line with the hypothesis that female gender may confer some degree of protection against myocardial amyloid deposition (Rapezzi et al., 2008). The electrocardiographic and structural abnormalities were common in this population; however, ejection fraction was reduced in only 3 patients. This latter result is consistent with cardiac failure, like the presence of NT-proBNP>900 pg/ml.

From those patients with reduced ejection fraction or NT-proBNP>900 pg/ml, only 15 patients had a clinical condition consistent with cardiac failure, as described in the patients from South of Portugal (Conceicao and De Carvalho, 2007). This fact may be explained by their low level of physical activity associated with a mPND score≥2.

Anemia was present in only 9.0%, a different result from a previous study conducted in Unidade Corino de Andrade, where 24.8% of patients had anemia (Beirao et al., 2004). This study suggested that anemia in TTR-FAP patients is caused by defective erythropoietin production (Beirao et al., 2004). The distinct results from these studies may be explained by the different disease duration of both samples: in the previous study the patients had an average disease duration of 6 years (Beirao et al., 2004), while in our study the observation was at the moment of diagnosis, which had a mean delay of 3 years.

Renal dysfunction and the degree of proteinuria are correlated with heavy amyloid deposition in the glomeruli, arterioles, and medium vessels (Lobato et al., 1998). A significant proportion of patients shows some degree of renal dysfunction, either proteinuria and/or reduction of glomerular filtration rate. The most severe involvement was observed only in female patients. Two patients had a nephrotic proteinuria with severe renal dysfunction and three patients were on dialysis. These results are in accordance with previous studies, that confer increased risk for nephropathy in patients >40 years old and in females (Lobato et al., 2004). As previously stated, microalbuminuria represents a first stage of clinical amyloid nephropathy (Lobato and

Rocha, 2012). The patient with mild proteinuria must therefore be followed in order to detect progression of renal disease.

Diagnosis of TTR-FAP remains difficult and requires expertise and adequate diagnostic methods. The mean delay between the first symptoms and diagnosis is 2.7 years, as described in Japanese (Koike et al., 2012), Italian (Verona) (Cappellari et al., 2011), Swedish (Suhr et al., 2009) and French (Mariani et al., 2015) population but better than those from Germany, where the delay is about 6.1 years (Dohrn et al., 2013), and from Italian (Rome) experience, where the delay is 4.3 years (Luigetti et al., 2013). There are several reasons to explain this delayed diagnosis such as the absence of family history and the variable phenotypes (from isolated ocular manifestations to typical polyneuropathy). Late-AO, up to 80 years of age, is also associated with multiple co-morbidities, making the diagnosis of this systemic disorder more difficult. The influence of having family history at the diagnostic delay is confirmed in this study: patients with family history had a delay of 2.3 years, while patients without family history had a delay of 3.5 years. This delay in diagnosis could be explained by the lack of awareness among patients without family history and among their physicians.

This study has some limitations, namely its retrospective nature, the multiplicity of clinical evaluators and the absence of follow-up evaluations. Despite that, we were able to show some important characteristics of these patients confirming the similarities with other late-onset series but also some important differences. The cause of these contrasting clinical features may be partially related to genetic heterogeneity, but they are also present when we compare patients with the same Val30Met mutation. This suggests a partial influence of the ethnic background, through candidate genes that alter disease pathways and have a role as genetic modifiers (Santos et al., 2017).

Further studies with a larger population and observation of disease progression as well as evaluation of the impact of different factors in the distribution of AO, along with a comparison between late- and early-onset TTR-FAP patients would help to better understand the natural history of this condition.

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